THE EMBRYOLOGY OF *EPALTES AUSTRALIS* LESS. (COMPOSITAE)

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Synopsis

The capitula of *E. australis* are heterogamous and, although all the florets are tubular, those of the outermost seven or eight whorls are filiform and female. Along any radius of the capitulum, the anthers develop in acropetal succession whereas the ovules form and mature in a basipetal manner.

Microsporogenesis is regular and the pollen is three-celled when shed.

Embryo sac formation follows the Polygonum type but only two antipodal cells are formed and the micropylar cell is binucleate. No secondary multiplication of antipodals occurs and the cells persist into post-fertilization stages. Fusion of the polar nuclei occurs immediately before fertilization and after the premature degeneration of the synergids.

Endosperm formation is *ab initio* Cellular and embryogeny conforms to the Asterad type.

Introduction

Of the three species of *Epaltes* endemic to Australia, *E. australis* is the most widely distributed and, according to Black (1957), it occurs in all states except Western Australia. It has also been reported from Formosa where it was possibly introduced in Australian wheat or wool.

The plant is herbaceous with procumbent stems which may reach 30 cm. in length and the hemispherical capitula are borne on short pedicels in the axils of the obovate leaves.

No member of this genus has previously been the subject of an embryological investigation although its component species are endemic to tropical Africa, Mexico, Brazil, tropical Asia and China, as well as Australia.

Materials and Methods

The material on which this investigation was based was collected from 40 miles east of Wanaaring, far western New South Wales, and fixed in F.A.A. by Professor N. C. W. Beadle of the University of New England.

After embedding, sections were cut at 8–10μ and stained with Delafield's haematoxylin and Johansen's safranin.

Floral Morphology

The capitula are hemispherical and heterogamous and the naked receptacle is slightly concave at maturity. The florets are all tubular and do not exceed the ovate, obtuse, involucral bracts, their number varying with the size of the capitulum. According to Black (1957), about 100 female florets surround the 8–25 bisexual disc florets but, in the Wanaaring material,

the ratio of female to bisexual florets was approximately 12:1 in a total of up to 400 florets per capitulum.

The filiform female florets occupy the outermost 7 or 8 whorls and, at maturity, the stylar arms protrude through the small aperture at the apex of the corolla tube (Text-fig. 1). Their outer surfaces are finely papillate and no definite stigmatic lines could be identified at their margins (Text-fig. 3).

The bisexual disc florets are shortly 4-lobed (Text-fig. 2) and the filaments of the four epipetalous stamens are inserted near the base of the corolla tube. Although the anthers are closely associated, they do not cohere into an anther tube. The stylar arms are lanceolate and taper sharply into the slender apices (Text-fig. 4). Finely papillate stigmatic lines occupy their margins and the large papillae on their outer surfaces are continuous with those on the distal end of the style proper. A ring-like nectary surrounds the base of the style and has no counterpart in the female florets.

The fruits of the bisexual florets are slightly broader than those of the females but both types are longitudinally ribbed and bear a microscopic rim which represents the pappus (Text-figs 1–2).

Microsporangium

The anthers are tetrasporangiate with sterile apices and are proximally tailed. Within the epidermis the fully formed anther wall is made up of endothecium, middle layer and tapetum and its method of formation follows the Dicotyledonous type (Davis, 1966). The cells of the amoeboid tapetum become multinucleate during meiosis in the adjacent microspore mother cells, and this may be accompanied by nuclear fusion. When the microspore tetrads are formed, the tapetal cells are 2- or 4-nucleate and, on breakdown of the tetrads, periplasmodium formation occurs.

Microsporogenesis and male gametogenesis follow the same sequence of events as described in *Podolepis jaceoides* (Davis, 1961) and the pollen grains are 3-celled when shed after the longitudinal dehiscence of the anther.

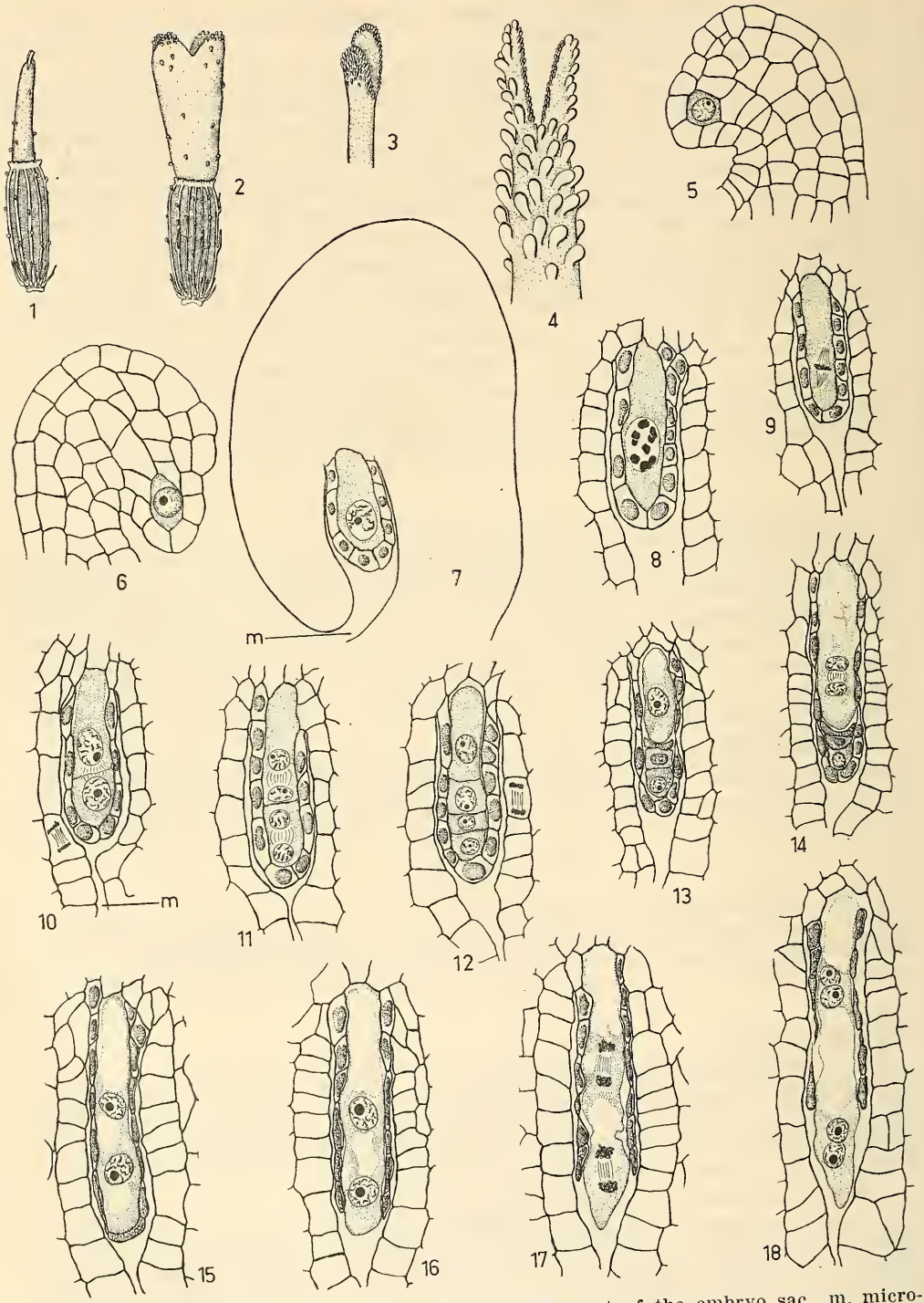
Megaspornagium

The initially atropous ovule becomes anatropous with the development of the single massive integument which overgrows the nucellus until it meets the funicle (Text-figs 5–7). As in other Compositae, the ovule is tenuinucellar and arises from the base of the inferior ovary.

Megasporogenesis

An archesporial cell differentiates at the apex of the nucellus in a sub-epidermal position and gives rise directly to the megaspore mother cell (Text-figs 5–7). The enlargement of this cell is accompanied by active cytoplasmic synthesis and it is accommodated in the nucellar lobe by anticlinal divisions of the overlying nucellar epidermal cells.

When the growth of the integument is completed, meiosis is initiated in the megaspore mother cell whose nucleus is situated towards the micropylar pole (Text-figs 8–9). Cytokinesis after meiosis 1 is therefore unequal and the chalazal dyad cell is larger than its micropylar counterpart (Text-fig. 10). Meiosis 11 takes place simultaneously in both dyad cells (Text-fig. 11) and is followed by wall formation but, whereas the two micropylar megaspores are the same size, the chalazal dyad cell divides unequally and the chalazal megaspore of the linear tetrad is the largest (Text-fig. 12). Although all the



Text-figs 1–18.—Floral morphology and development of the embryo sac. m, micropyle. Text-figs 1, 2 × 17; 3, 4 × 100; 5, 6, 8–18 × 600; 7 × 433.

megaspores may develop vacuoles and, consequently, are said to germinate, only the chalazal megaspore increases in size and the three non-functional megaspores degenerate (Text-fig. 13).

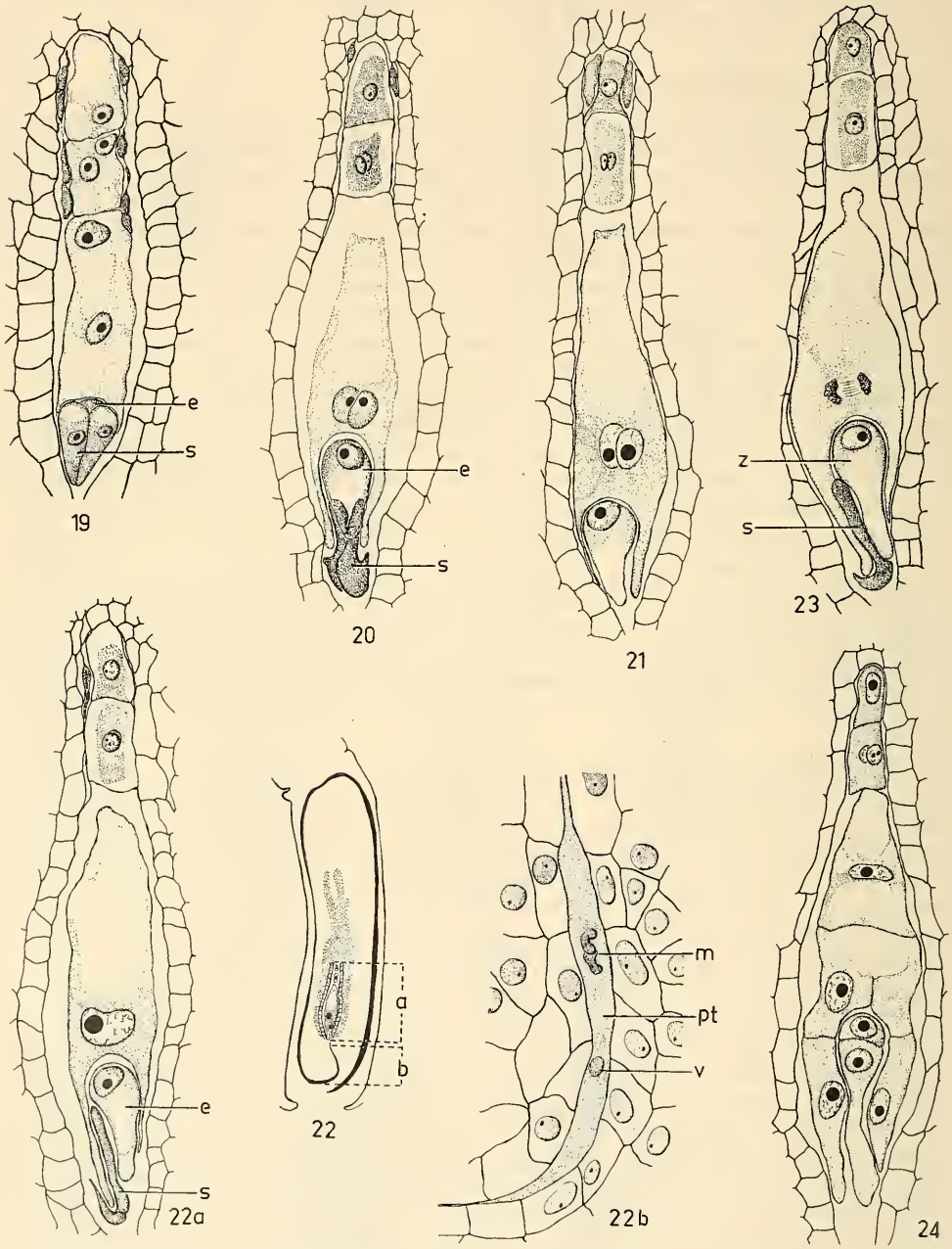
Embryo sac formation

When the functional megaspore enlarges, the nucellar epidermis becomes tangentially stretched and its cells do not divide anticlinally. The transition from the functional megaspore stage to the 1-nucleate embryo sac is purely a matter of size increase and is not associated with any anatomical characteristics. In most Compositae, the 1-nucleate embryo sac fills the nucellar lobe after the dissolution of the non-functional megaspores, but in *E. australis* the latter are still in evidence when the nucleus of the embryo sac divides (Text-fig. 14). A vacuole forms between the daughter nuclei and, following the eventual absorption of the crushed non-functional megaspores, the 2-nucleate embryo sac comes into direct contact throughout its length with the nucellar epidermis (Text-fig. 15). Further enlargement of the embryo sac results in its apex penetrating the nucellar epidermis and taking up its position at the throat of the micropyle (Text-fig. 16). Although the cell contents of the nucellar epidermis are in a degenerated condition, the layer itself persists until maturity of the embryo sac and its remains can be recognised internal to the endothelium.

The second embryo sac mitosis leads to the 4-nucleate stage in which the nuclei occupy the 2+2 configuration, the two pairs being separated by a large vacuole which was formed after the preceding nuclear division (Text-figs 17–18). The third and last embryo sac mitosis, as well as the following free 8-nucleate stage, were not observed but, after cytokinesis, differentiation occurs and the development of the embryo sac is, therefore, of the Polygonum type.

The differentiated embryo sac is 6-celled (Text-fig. 19), due to the formation of only two antipodal cells, of which the micropylar is invariably 2-nucleate. In the newly-formed embryo sac, the antipodal cells are vacuolate and their cytoplasm stains lightly but, at maturity, the vacuoles disappear and each cell becomes filled with dense cytoplasm. No nuclear divisions occur and the two cells remain in this condition until they degenerate when embryogeny is well advanced. In the endosperm mother cell, the two polar nuclei initially occupy opposite poles and are separated by the large vacuole which has persisted since the 2-nucleate stage of the embryo sac. The chalazal polar nucleus then migrates to the micropylar portion of the cell where it becomes closely associated with its micropylar counterpart. Fusion of the polar nuclei to form a secondary nucleus is delayed until just before fertilization. As in other Compositae, the three cells of the egg apparatus are partially enclosed by the hood-like micropylar portion of the endosperm mother cell. When the synergids are first formed, they are slender basally vacuolate cells which taper towards their apices at the throat of the micropyle. However, during their degeneration, each develops a lateral fold which appears hook-like in section (Text-fig. 20). In *Epaltes australis* the breakdown of the synergids is premature in that it commences before the formation of the secondary nucleus and is not associated with the entrance of the pollen tube into the embryo sac. The egg is an elongated cell which is deeply enfolded by the endosperm mother cell and its micropylar half is occupied by a large vacuole (Text-figs 20–21).

The micropylar chamber, which contains the nucellar lobe, is bounded by the innermost cell layer of both the integument and the raphe. These cells



Text-figs 19–24.—Mature embryo sac, fertilization and endosperm formation. e, egg; m, male gametes; pt, pollen tube in micropyle; s, sygergids; v, vegetative nucleus; z, zygote. Text-figs 19 × 600; 20, 21, 23, 24 × 433; 22 × 67; 22a, 22b × 1000.

divide only anticlinally (Text-figs 10, 12) and, by the time the megaspore mother cell enters meiosis, they have formed the endothelium. In cell arrangement and staining capacity, this cell layer is sharply demarcated from the remainder of the integumentary cells and it reaches its maximum development at maturity of the embryo sac. Its cells show signs of degeneration after endosperm formation has been initiated and it can be distinguished only by its position by the time the young embryo has become heart-shaped. The development of an endothelium is invariable in the Compositae and it is a character of tenuinucellar ovules in general, where the embryo sac comes into direct contact with the integument after the breakdown of the nucellus. Circumstantial evidence indicates that it may play a part in the nutrition of the embryo sac by producing enzymes which digest the contents of the integumentary cells external to it. In text-figure 22 this process of digestion is well advanced and a region of empty collapsed cells extends outwards from the endothelium, within which is the mature embryo sac.

Fertilization

Syngamy was not observed, but in one ovule (Text-fig. 22) in which a secondary nucleus was present and the synergids had degenerated (Text-fig. 22a), a pollen tube was present in the micropyle and contained two closely associated vermiform male gametes as well as the vegetative nucleus from the pollen grain (Text-fig. 22b).

Post-fertilization Events

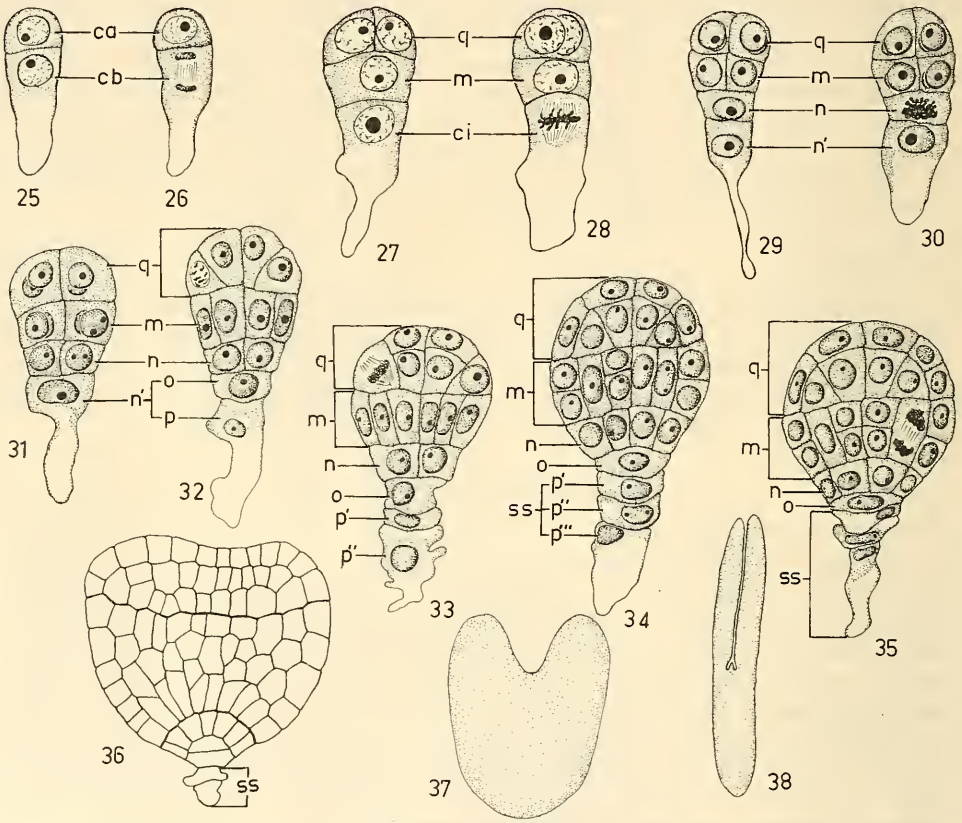
Endosperm formation

Division of the primary endosperm nucleus precedes that of the zygote. The mitotic spindle lies at right angles to the long axis of the embryo sac and a wall is laid down across the telophase spindle (Text-fig. 23). Endosperm formation is therefore of the *ab initio* Cellular type and subsequent divisions follow rapidly. By the time the proembryo is 2-celled, endosperm formation is well advanced (Text-fig. 24) but there is no evidence of its digestion before the embryo has reached the stage shown in text-figure 35. When the embryo is fully grown all the endosperm has been utilized with the exception of a layer of regular thick-walled cells within the remains of the integument. The seed, therefore, is not strictly exalbuminous.

Embryogeny

After formation of five or six endosperm cells, the zygote divides transversely to form the superposed cells *ca* and *cb* of the two-celled proembryo (Text-fig. 25). Vertical division of *ca* gives rise to the tier *q* and *cb* divides transversely to form the cells *m* and *ci* of the four-celled proembryo (Text-figs 26–27). A vertical division in *q* and *m* converts the first into a quadrant and the second into two juxtaposed cells, while *ci* divides transversely to form *n* and *n′* (Text-figs 28–29). The eight-celled proembryo is now made up of four tiers of cells, *q*, *m*, *n* and *n′*; further vertical divisions transform *m* into a quadrant and replace the single cell of *n* by two juxtaposed cells (Text-figs 30–31). This is the final stage of the proembryo and, after transverse division of *n′* into *o* and *p*, the four-tiered embryo proper is delimited from the suspensor which originates from *p*. Because *ca* undergoes a vertical division and cells derived from both *ca* and *cb* take part in the formation of the embryo proper, the embryogeny of *E. australis* conforms to the Asterad

type. The quadrant *q* now becomes an octant and a periclinal division of each cell of *m* cuts off a central quadrant (Text-fig. 32). The cells of *q* divide periclinally, further vertical and periclinal divisions occur in *m*, *n* becomes a quadrant and *p* forms *p′* and *p″* (Text-fig. 33). Transverse divisions cause *m* to become two-tiered and the three-celled suspensor (ss) is established (Text-figs 34–35). The embryo proper has now assumed a globular form and



Text-figs 25–38.—Embryogeny. (Lettering follows the system of Souège.) Text-figs 25–35 × 600; 36 × 433; 37 × 270; 38 × 50.

the suspensor cells become highly vacuolate and of irregular outline. Their contents disappear and, although the suspensor can still be identified when the embryo becomes heart-shaped (Text-fig. 36), no trace of it remains after the initiation of the cotyledons (Text-figs 37–38).

Maturation Gradients in the Capitulum

The capitulum being a racemose inflorescence, its florets mature in acropetal succession and the youngest occupy a central position. There is therefore a gradient expressed in the maturation of both anthers and ovules along any radius. In *E. australis*, however, although the stages of microsporogenesis were found to conform to this centripetal pattern, a reverse gradient was observed in the development of the ovules. In all the capitula examined the central florets contained the youngest anthers and the most mature ovules,

whereas the peripheral female florets contained the youngest ovules. This phenomenon of opposite maturation gradients in the male and female reproductive structures of a capitulum has not been reported previously and, in order to investigate it more closely, the ovules of florets occupying three sites in median sections of five capitula were compared:

	Central Bisexual Floret	Innermost Female Floret	Outermost Female Floret
1.	Growth of integument almost completed	Integument development initiated	Ovule atropous
2.	Megaspore tetrad	Meiosis II	Meiosis I
3.	2-Nucleate embryo sac	Functional megaspore	Megaspore tetrad
4.	4-Nucleate embryo sac	First embryo sac mitosis	Functional megaspore
5.	2-Cellerd poembryo	2-Celled proembryo	Mature embryo sac

Due to their earlier maturation, the florets at the centre of the capitulum are the first to be pollinated and proembryos are found in their ovules before fertilization occurs in the peripheral florets. During embryogeny, the maturation gradient becomes less apparent and, in the infructescence, the ovaries of all florets contain a seed with a fully developed embryo.

Discussion

Although embryo sac formation follows the Polygonum type, cytokinesis is such that invariably the chalazal antipodal nucleus is enclosed in one cell and the remaining two in the second. This nuclear disposition is in agreement with all other reports in the Compositae where there are only two antipodal cells and there is no record of the chalazal cell ever being the binucleate one. Variation does occur, however, in the behaviour of these cells. For example, in *Synedrella nodiflora* (Banerji and Pal, 1955) and *Tridax trilobata* (Hjelmqvist, 1951) fusion may occur between the nuclei in the micropylar cell so that both cells are then uninucleate, and in *Bidens biternata* (Deshpande, 1964*b*) and *Gerbera jamesonii* (Maheswari Devi, 1957) the nuclei divide and each cell becomes multinucleate. The commonest condition, however, is where nuclear division is accompanied by cell division. This secondary multiplication of antipodal cells has been reported by Harling (1951) in *Chrysanthemum arcticum*, *Erigeron canadensis* and *Matricaria globifera* and later (1954) in *Vittadinia triloba*. In *Epaltes australis* no embryo sac was observed with more than two antipodal cells and, although the two nuclei in the micropylar cell were closely associated, fusion did not occur. In this respect, this species is similar to *Chrysanthemum flosculum* (Harling, 1951), *Flaveria repanda* (Misra, 1957), *Matricaria chamomilla* (Harling, 1951), *Senecio glutinosus* (Afzelius, 1924), *Tridax procumbens* (Maheshwari and Roy, 1952), *Volutarella ramosa* (Deshpande, 1964a) and *Wedelia calendulacea* (Ghosh, 1962) although the occasional occurrence of three antipodal cells is reported in these examples.

The premature degeneration of the synergids is an unusual feature of the embryology of *E. australis*. This appears to be an autonymous breakdown of the cells because it occurs before the entry of the pollen tube and while the embryo sac is still immature. No comparable examples of this phenomenon have been traced in the literature.

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